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TOCOPHEROL SUPPLEMENTATION ON STOCKING DENSITY OF BROILER: EFFECT ON PERFORMANCE CHARACTERISTICS AND SERUM ENZYMES

[SUPLEMENTACIÓN CON TOCOFEROL Y DENSIDAD DE CRIANZA EN AVES DE ENGORDA: EFECTO SOBRE DESEMPEÑO Y ENZIMAS SÉRICAS]

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SUMMARY

A total of two hundred and seventy day-old Arbor Acre strain of broiler chicks were used for this research. Five treatment: T1 - positive control (10 birds/m^2), T2 - negative control, T3, T4 and T5 had 20 birds/m^2. T1 and T2 had no supplementation with vitamin E (d1-α-tocopheryl acetate). T3, T4 and T5 had 50mg/kg, 100mg/kg and 150 mg/kg vitamin E supplementations respectively. Feed intake, feed conversion ratio (FCR) and weight gain and serum enzymes (Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)) were determined. There were no significant changes in the weight gain and final weight of the birds fed the different dietary treatments. The feed intake increased significantly in birds fed T2 (1.91kg) and compared to their counterpart on vitamin E supplementation (from 1.58 to 1.60 kg). However, FCR of birds on diets T1 (2.50), T4 (2.77) and T5 (2.50) was similar (P>0.05). The total protein and Aspartate Aminotransferase (AST) values were neither affected by increased stocking density nor with or without vitamin E supplementation. However, increased stocking density without vitamin E supplementation (T2) (0.97 U.I/l) resulted in a significant reduction in the albumin values. Although ALT values increased significantly with increase in vitamin E supplementation, the birds on dietary T1 (8.00 U.I/l) had similar level of ALT with their counterpart on T4 (7.50U.I/l) and T5 (8.50 U.I/l). In conclusion broiler chicks could be stocked up to 20 birds/m^2 only if the diet is supplemented with 100mg/kg vitamin E.

Key words: Stock density; vitamin E; serum enzymes; broilers

RESUMEN

Un total de ciento setenta y dos aves de engorda (Arbor acre) fueron empleadas en cinco tratamientos: T1 – control positivo (10 aves/m^2), T2 – negative control, T3, T4 y T5 tuvieron 20 aves/m^2. T1 y T2 no fueron suplementados con vitamina E (dl-a-acetato de tocoferol). T3, T4 y T5 fueron suplementados con 50, 100 y 150 mg/kg vitamina E respectivamente. Las variables de respuesta fueron: consumo de alimento, conversión de alimento (CA), ganancia de peso y las enzimas séricas Aspartato aminotransferasa (AST) y alanina aminotransferasa (ALT). No hubo efecto sobre ganancia de peso y peso final de las aves. El consumo de alimento fue mayor (P<0.05) en T2 (1.97 kg) en comparación con T3 y T4 consumieron 1.58 y 1.60 kg respectivamente. La CA fue similar para T1, T4 y T5 (2.5, 2.77 y 2.5 respectivamente) (P>0.05). La proteína total y AST no fueron afectados por la densidad o la suplementación con vitamina E. Sin embargo, una mayor densidad sin suplementación con vitamina E (T2) resultó en una reducción de los valores de albúmina. Aunque ALT se incrementó (P<0.05) con la suplementación de vitamina E, las aves en T1 tuvieron valores similares que aquellas aves en T4 y T5. En conclusión, las aves de engorda pudieran ser mantenidas a densidades de hasta 20 aves/m^2 si cuentan con una suplementación de vitamina E de 100 mg/kg.

Palabras clave: densidad; vitamina E; enzimas séricas; pollos de engorda.

INTRODUCTION

Stress in broiler production is not only restricted to heat (high ambient temperature), but also physiological stress (as a result of increasing stocking density), nutritional stress (imbalance in the nutrient requirement) and vaccination stress etc. Increasing stocking density of broilers is a management practice used for reducing cost associated with labour, housing and equipments. However, over-crowding of broilers can lead to reductions in performance (Shanawany, 1988). Broiler performance and health can be influenced by very high stocking density (Webster, 1990) thereby it is important to ensure that adequate floor space is available for each bird (Al-Homidan, 2001). If the stocking density is too high, the temperature may rise dangerously since there will be more metabolic heat being added to the house air than was planned for.

Poultry farmers often increase stock density with the aim of increasing profit but this always result in the build up of heat and consequently leading to heat stress. Several methods are available to alleviate the effect of high environmental temperature and increased stocking density on performance of poultry. Since it is expensive to cool animal buildings, such methods are focused mostly on the dietary manipulation. In this respect, vitamin E is used in the poultry diet because of the reported benefits of vitamin E supplementation to laying hens during heat stress (Whitehead *et al*., 1998; Bollengier-Lee *et al.*, 1998, 1999; Sahin *et al*., 2001), also because of the fact that vitamin E levels is reduced during heat stress (Feenster, 1985; Whitehead *et al*., 1998; Boliengier-Lee *et al*., 1999; Sahin *et al*., 2001, 2002) .

Vitamin E has been recognized as an essential nutrient for growth and health of all species of animals (McDowell, 1989). The diverse roles of vitamin E are due to its involvement in nutritional myopathy, prostaglandin biosynthesis and immune responsiveness (Lin *et al*., 1996). Asghar *et al*. (1991) recorded improvements in animal performance when pigs were supplemented with 100 mg vitamin E/kg of feed. One of the most important properties of vitamin E is its antioxidant function. When animals fed diets rich in unsaturated fatty acids which are susceptible to peroxidation the vitamin E deficiency is augmented (McDowell, 1989). Supplementation of animal diets with tocopherols increases the content of this natural antioxidant in animal food products and prevents lipid peroxidation in broiler meat (Ajuyah *et al.,* 1993). Vitamin E is known to be a lipid component of biological membranes and is considered a major chain-breaking antioxidant (Halli-well and Gutteridge, 1989). Vitamin E is mainly found in the hydrocarbon part of membrane lipid bilayer towards the membrane interface and in close proximity to oxidase enzymes which initiate the production of free radicals (Putnam and Comben, 1987; McDowell, 1989; Packer, 1991). Vitamin E, therefore, protects cells and tissues from oxidative damage induced by free radicals (Gallo-Torres, 1980). Sahin and Kucuk, (2001) observed that supplemental vitamin E significantly alleviated the heat stress-related decrease in performance suggesting additional vitamin E supplementation into diets may be necessary under heat stress conditions in Japanese quails. Supplementing vitamin E to broilers is also important to human health in terms of consuming healthier poultry meat products.

Therefore, the objective of this study was to evaluate the effects of optimal dose of vitamin E supplementation on performance and serum enzyme concentrations of broilers reared under increased stocking density.

MATERIALS AND METHODS

Total of Two hundred and seventy, day-old Arbor Acre strain of broiler chicks were used for this research. The study was carried out at the Teaching and Research Farm of the University of Ibadan, Ibadan for a period of four weeks. The birds were randomly divided into five treatment groups of total of 30 birds in Treatment 1 (positive control) while those in Treatments 2 (negative control), 3, 4 and 5 had 60 birds per treatment. Birds in Treatment 1 were further sub-divided into three replicates with 10 birds per replicate while their counterparts in Treatments 2 to 5 were subdivided into 20 birds per replicate. All pens were bedded with a wood-shavings litter and equipped with feeders and waterers. Birds fed dietary treatment 1 had a spacing of 10 birds/m^2 (0.1m^2/bird) without Vitamin E (d1-α-tocopheryl acetate) supplementation (positive control) while those in treatment 2 had a stocking density of 20 birds/m^2 without Vitamin E (negative control). However, birds on dietary treatments 3 to 5 had a stocking density of 20 birds/m^2 (0.05m^2/bird) supplemented with 50mg/kg, 100mg/kg and 150 mg/kg respectively.

At the end of day 28, 9 birds randomly chosen from each treatment (3 birds per each replicate) were slaughtered and blood was collected. Blood samples were centrifuged at 3 000 × *g* for 10 min and serum was collected and stored for later analysis. Total protein, albumin, and serum enzyme activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were measured using a biochemical analyzer kit (Olympus AU-600 System).

All the data including the serum parameters were subjected to statistical analysis of variance (ANOVA) procedure of SAS, 1999. The design of the experiment was of the completely randomized design (CRD)
The statistical model:

$X_{IJ} = \mu + \alpha_t + e_{IJ}$
μ = grand mean
α_t = treatment effect
e_{IJ} = error terms

The basal composition of the experimental diet is shown Table 1. The birds were fed their respective experimental diets *ad-libitum.* At weekly intervals, feed intake and body weight were determined. Weight gain and feed efficiency (FCR) of birds were then calculated.

RESULTS

There were no significant changes in the weight gain and final weight of the birds fed the different dietary treatments (Table 2). The feed intake increased significantly in birds fed T2 (1.91kg) (negative control) and those compared to their counterpart on Vitamin E supplementation (from 1.58 to 1.60 kg). However, FCR of birds fed diets T1 (2.50), T4 (2.77) and T5 (2.50) are significantly similar.

Table 3 showed the differences in the serum metabolites and serum enzymes of broiler birds fed different levels of Vitamin E supplementation. The total protein and Aspartate Aminotransferase (AST) values for the birds on the different treatments ware not significantly affected by neither high stocking density nor vitamin E supplementation. Nevertheless, the albumin values increased significantly in birds fed the different levels of Vitamin E supplemented diets compared with birds that are sparsely spaced (10 birds/m^2; T1, positive control) (1.12 U.I/l). However, increased stocking density without Vitamin E supplementation (20 birds/m^2; T2, negative control) (0.97 U.I/l) resulted in a significantly reduction in the albumin values. Although ALT values increased significantly with increase in Vitamin E supplementation, the birds on dietary Treatment T1 (10 birds/m^2) (8.00 U.I/l) had similar level of ALT with their counterparts on T4 and T5 (7.50 and 8.50 U.I/l respectively).

DISCUSSION

In the present study, vitamin E supplementation at 100mg/kg in an increased stock density (0.2m^2/bird) compared favourably with birds on the positive control (0.1m^2/bird) in the efficiency of feed utilisation. The increase in the feed conversion ratios of birds in dietary treatments 2 and 3 resulted from increasing stocking density could be attributed to the increase in stress resulting from competition for feed, and water, increase of house temperature, microbial activity and ammonia production.

Table 1. Gross composition of experimental diet

Ingredients (kg)	TI (Positive control)	T2 (negative control)	T3 (50mg/kg Vitamin E)	T4 (100mg/kg Vitamin E)	T5 (150mg/kg Vitamin E)
Maize	58.00	58.00	58.00	58.00	58.00
Groundnut Cake	21.00	21.00	21.00	21.00	21.00
Palm kernel cake	1.00	1.00	1.00	1.00	1.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Soyabean meal	14.60	14.60	14.60	14.60	14.60
Bone meal	2.40	2.40	2.40	2.40	2.40
Premix (Broiler starter)	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20
Vitamin E (mg/kg)	0.00	0.00	50.00	100.00	150.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrient					
Crude Protein (%)	23.00	23.00	23.00	23.00	23.00
Metabolisable Energy (kcal/kg ME)	3,019.27	3,019.27	3,019.27	3,019.27	3,019.27
Crude fibre (%)	3.30	3.30	3.30	3.30	3.30
Calcium (%)	1.05	1.05	1.05	1.05	1.05

T1=10 birds/m^2; T2= 20 birds/m^2 ; T3= 20 birds/m^2 + 50mg/kg vit E; T4= 20 birds/m^2 + 100mg/kg vit E; T5= 20 birds/m^2 + 150mg/kg vit E

Table 2. Effect of stocking density and different levels of vitamin supplementation on performance characteristics of broiler chicks.

Dietary treatments						
Parameter (kg)	TI (Positive control)	T2 (negative control)	T3 (50mg/kg Vitamin E)	T4 (100mg/kg Vitamin E)	T5 (150mg/kg Vitamin E)	SEM
Initial weight	0.12	0.10	0.10	0.11	0.11	
Final Weight	0.75	0.68	0.68	0.68	0.78	0.13
Weight gain	0.63	0.58	0.58	0.57	0.64	0.43
Feed intake	1.58^{c}	1.91^{a}	1.76^{b}	1.58^{c}	1.60^{c}	1.03
Feed conversion Ratio (FCR)	2.50^{b}	3.29^{a}	3.03^{a}	2.77^{b}	2.50^{b}	1.56

^{abc}: Means on same row with different superscripts differ significantly (P<0.05)
T1=10 birds/m^2; T2=20 birds/m^2 ; T3=20 birds/m^2 + 50mg/kg vit E; T4=20 birds/m^2 + 100mg/kg vit E; T5= 20 birds/m^2 + 150mg/kg vit E

Table 3. Effect of stocking density and different levels of vitamin supplementation on serum metabolites and enzymes of broiler chicks.

Dietary treatments						
Parameter	TI (Positive control)	T2 (negative control)	T3 (50mg/kg Vitamin E)	T4 (100mg/kg Vitamin E)	T5 (150mg/kg Vitamin E)	SEM
Total Protein (g/dl)	4.26	3.56	3.71	3.86	4.18	1.20
Albumin (g/dl)	1.12^{bc}	0.97^{c}	1.27^{ab}	1.40^{a}	1.42^{a}	1.05
Aspartate Aminotransferase (AST) (U.I/l)	50.00	40.00	47.50	45.00	50.00	4.98
Alanine Aminotransferase (ALT) (U.I/l)	8.00^{a}	5.00^{c}	6.50^{b}	7.50^{a}	8.50^{a}	1.45

^{abc}: Means on same row with different superscripts differ significantly (P<0.05)
T1= 10 birds/m^2; T2=20 birds/m^2 ; T3=20 birds/m^2 + 50mg/kg vit E; T4=20 birds/m^2 + 100mg/kg vit E; T5=20 birds/m^2 + 150mg/kg vit E

Kennedy et al. (1992) examined the productivity of 168 broiler flocks fed diets containing either 50 mg/kg or 180 mg/kg dietary vitamin E. The authors reported that at the greater level of vitamin E supplement, productivity was 8.4% greater as a result of improvements in both FCR and higher average weight gain. Similarly, Sahin and Kucuk (2001) found that dietary vitamin E inclusions resulted in a greater performance in Japanese quails reared under heat stress (34°C). The vitamin E supplementation was able to ameliorate the effect of heat stress that would have resulted from the overstocking. Ushakova *et al.*, 1996 showed that dietary supplements of vitamin E can modify gene expression induced by heat shock *in vivo* and have a protective role against oxidative stress by enhancing the level of endogenous antioxidants and inducing heat shock protein (hsp)-70 gene expression. Organisms respond to elevated temperatures and physiological stresses by an increase in the synthesis of heat shock proteins (Hsp) or stress proteins. According to Sahin *et al*. (2002), under high environmental temperature, the expression of genes for Hsp will be enhanced and the proteins will accumulate in cells. The authors further reported that vitamin E supplementation in heat stressed broiler house resulted in better performance, perhaps due to increased Hsp synthesis. The cells with increased Hsp exhibit tolerance against the additional stress. The increased FCR in vitamin supplemented group is also in agreement with the earlier reports of Villar *et al.* (2002) who reported that feed efficiency increased statistically with vitamin supplementation.

Contrary to the result obtained, Al- Homidian (2001) revealed that there was no significant difference in broiler performance due to stocking density However, the study revealed that increased stocking density reduced feed utilisation in broiler as shown by birds fed dietary Treatment 2 (0.05m^2/bird). The study also clearly showed that even if the stocking density of broiler is doubled and metabolic heat increases leading to heat stress in broiler house, supplementation of the diet with vitamin E will result in an improve production.

The study further revealed that there were no negative effect of stocking density on the serum total protein, and AST. The decrease observed in the ALT and albumin contents of birds in T2 (20birds/m^2 - vit E) could be due to the oxidative ability that would have been mitigated if vit E has been added. Lin et al (1996) observed that plasma AST was significantly improved when green tea (substance containing antioxidant) was included in the diet of broiler. The authors attributed the improvement to the antioxidative effect of the green tea. However, El Deek and Al-Harthi, (2004) indicated that green tea addition in the diets of broiler chicks stocked at 10 birds/m^2 and 18 birds/m^2 increased the activity of plasma AST compared to those on control group.

It is clear that increase stocking density supplemented with vitamin E improved the plasma constituents of broiler chicks as judged by plasma AST and ALT during the experimental period.

CONCLUSION

In conclusion the result showed that for improvement in the feed intake and weight gain of broiler chicks stocked at 20 birds/m^2 vitamin E supplementation is essential. The study further showed that 100mg/kg vit E supplementation improved both serum metabolites and performance of broiler.

REFERENCES

Al-Homidan, A. A 2001. The effect of temperature and stocking density on broiler performance and ammonia production. Egypt Poult. Sci., 21: 1121-1137

Ajuyah, A.O; Hardin R.T. and Sim J.S. 1993: Effect of dietary full-fat flax seed with and without antioxidant on the fatty acid composition of major lipid classes of chicken meats.

Asghar, A; Gray, J. I; Booren, A. M; Gomaa, E. A; Abouzied, M. M. and Miller, E. F. 1991. Effects of supranutritional dietary vitamin E levels on subcellular deposition of α-tocopherol in the muscle and on pork quality. Journal of the Science of Food and Agriculture, 57: 31–41.

Bollengier-Lee S; Mitchell M.A; Utomo, D.B; Williams, P.E.V. and Whitehead, C.C. 1998. Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. British Poultry Science. 39, 106–112.

Bollengier-Lee S; Williams P.E.V. and Whitehead, C.C. 1999: Optimal dietary concentration of vitamin E for alleviating the effect of heat stress on egg production in laying hens. British Poultry Science. 40, 102–107.

El- Deek, A.A. and. Al-Harthi, M.A. 2004, Responses of Mordern Broiler Chicks to Stocking Density, Green Tea, Commercial Multi Enzymes and Their Interactions on Productive Performance, Carcass Characteristics, Liver Composition and Plasma Constituents. International Journal of Poultry Science 3: 635-645.

El- Deek, A.A; Al-Harthi, M.A; Attia, Y.A. and Maysa-Hannfy M. 2003. Effect of anise (Pimpinella anisum), fennel (Foeniculum vulgare) and ginger (Zingiber officinale Roscoe) on growth performance, carcass criteria and meat quality of broilers. Archive Fur Geflugelkunde, 67: 92-96

Feenster, R. 1985: High temperatures decrease vitamin utilization. Misset Poultry, 38–41.

Gallo-Torres D.C. (1980): Absorption, blood transport and metabolism of vitamin E. In: Maclin L.J. (ed.): A Comprehensive Treatise. Marcel Dekker, New York. 170–267.

Halliwell, B. and Gutteridge J.M.C. 1989. Free Radicals in Biology and Medicine. 2nd ed. Oxford University Press, New York.

Kennedy, D.G; Rice, D.A; Bruce, D.W; Goodall, E.A. and McIlroy S.G. 1992. Economic effects of increased vitamin E supplementation of broiler diets on commercial broiler production. British Poultry Science. 33, 1015–1023.

Lin, Y.L; Juan, I.M; Chen, Y.L; Liang, Y.C. and Lin, J.K. 1996. Composition of polyphenols in fresh tea leaves and associations of their oxygen- radical-absorbing capacity with antipoliferative actions in fibroblast cells. Jouurnal of Agriculture and Food Chemestry. 272: 1433-1436.

McDowell, L.R. 1989. Vitamins in Animal Nutrition – Comparative Aspects to Human Nutrition. In: Mc Dowell L.R. (ed.): Vitamin A and E. Academic Press, London. 10–52, 93–131.

Packer, L. 1991. Protective role of vitamin E in biological systems. Am. J. Clin. Nutr., *53*, 1050–1055.

Putnam, M.E. and Comben, N. 1987. Vitamin E. Vet. Rec., *121*, 541–545.

Sahin, K. and Kucuk, O. 2001. Effects of vitamin C and vitamin E on performance, digestion of nutrients, and carcass characteristics of Japanese quails reared under chronic heat stress (34°C). J. Anim. Physiol. Anim. Nutr., *85*, 335–342.

Sahin, K; Kucuk, O; Sahin, N. and Sari, M. 2002. Effects of vitamin C and vitamin E on lipid peroxidation status, some serum hormone, metabolite, and mineral concentrations of Japanese quails reared under heat stress (34°C). Int. J. Vitamin Nutr. Res., *71*, 91–100.

Sahin, K; Sahin N; Onderci, M; Yaralioglu, S. and Kucuk, O. 2001. Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. Vet. Med. – Czech, *46*, 140–144.

SAS Institute 1999. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC

Shanawany, M.M. 1988. Broiler performance under high stocking densities. British Poultry Science. 29: 43-52.

Ushakova, T; Melkoyan, H; Nikonova, L; Mudrik, N; Gogvadze, V; Zhukova, A; Gaziev, A.I. and Bradbury R. 1996. The effect of dietary supplements on gene expression in mice tissues. Free Radic. Biol. Med., *20*, 279–284.

Villar, P.G; Diaz, C.A; Avila, G.E; Guinzberg, R; Pablos, J.L. and Pina, E 2002. Effects of dietary Supplementation with vitamin C or vitamin E on growth performance in broilers. American Journal of Veterinary Research 63: 573-576.

Webster, A.J. 1990. Housing on respiratory disease in farm animals. Outlook on Agriculture, 19: 31-35

Whitehead, C.C; Bollengier-Lee S; Mitchell, M.A. and Williams, P.E.V. 1998. Vitamin E can alleviate the depression in egg production in heat stressed laying hens. In: Proc. of Spring Meeting, WPSA-UK Branch, Scarborough, 55–56.

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